Docket No.: AFMX-P02-038
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
McGall et al.

Application No.: 09/862571 Confirmation No.: 9482

Filed: May 21, 2001 Art Unit: 1631

For: METHODS FOR REDUCING NON-SPECIFIC BINDING TO AN OLIGONUCLEOTIDE ARRAY Examiner: M. L. Borin

APPEAL BRIEF

MS Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This Appeal Brief is submitted pursuant to the Notice of Appeal received in the U. S. Patent & Trademark Office on January 27, 2006, and in support of the appeal from the final rejections set forth in the Office Action mailed on July 25, 2005. The fee for filing a brief in support of an appeal is enclosed.

The fees required under 37 C.F.R. § 41.20(b)(2) are addressed in the accompanying Transmittal of Appeal Brief. A Petition for Extension of Time and the appropriate fee are also being filed concurrently.

This brief contains items under the following headings as required by 37 C.F.R. § 41.37 and M.P.E.P. § 1206:

I.	Real Party In Interest
II	Related Appeals and Interferences
III.	Status of Claims
IV.	Status of Amendments
V.	Summary of Claimed Subject Matter

I. REAL PARTY IN INTEREST

The real party in interest for this appeal is AFFYMETRIX, INC., the Assignee of the entire right, title and interest in the above-identified application.

II. RELATED APPEALS, INTERFERENCES, AND JUDICIAL PROCEEDINGS

There are no other appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

III. STATUS OF CLAIMS

A. Total Number of Claims in Application

There are 14 claims pending in application, 9 of which are presented for appeal, as shown in Section VIII.

B. Current Status of Claims

1. Claims canceled: 13-42

2. Claims withdrawn from consideration but not canceled: 4, 5, 8, 10 and 11 (incorrectly listed as 4, 5 and 8-11 in the Final Office Action)

3. Claims pending: 1-12, 43 and 44

4. Claims allowed: none

5. Claims rejected: 1-3, 6, 7, 9, 12, 43 and 44

C. Claims On Appeal

The claims on appeal are claims 1-3, 6, 7, 9, 12, 43 and 44.

IV. STATUS OF AMENDMENTS

Applicant filed an Amendment After Final Rejection on February 6, 2006. The Examiner responded to the Amendment After Final Rejection in an Advisory Action mailed February 28, 2006. In the Advisory Action, the Examiner indicated that Applicants' proposed amendments to claims 9 and 11 would not be entered. (The Amendment filed February 6, 2006 mistakenly indicated the status of claims 43 and 44 as "new;" instead, these claims should have been labeled as "previously presented," because they were added in the Amendment filed April 29, 2005 and entered by the Examiner.)

Accordingly, the claims listed in Appendix A do not incorporate the amendments to claim 9, as indicated in the paper filed (claim 11 is not shown because it has been withdrawn from consideration). Thus, the claims in Appendix A represent the pending claims on appeal as filed by Applicant on April 29, 2005.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claims under appeal are directed to a method for reducing non-specific binding of a molecule to an oligonucleotide array (page 2, lines 14-15 of the specification). In this method, a plurality of oligonucleotides having a terminal protecting group is produced on a surface having protected regions (page 2, line 29 through page 3, line 3). The protecting groups on one or more of the oligonucleotides are removed and replaced by a negatively charged phosphate residue (page 3, lines 3-8). The negatively charged phosphate residue can either be a single entity (as in claim 10) or a polyanion chain (as in claims 11 and 12) (page 3, lines 8-11). The method is particularly useful in preventing non-specific binding of a nucleic acid polymer to an oligonucleotide array (Examples 1 and 2).

VI. GROUNDS OF OBJECTION TO BE REVIEWED ON APPEAL

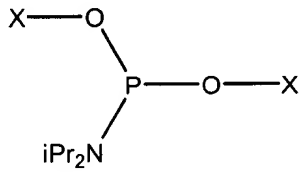
1. Whether claims 1-3, 6, 7, 9, 12, 43 and 44 are properly rejected under 35 U.S.C. § 112, second paragraph, even though the details of phosphoramidite chemistry are well-known to those skilled in the art and are not essential material.

2. Whether claims 1-3, 6, 7, 9, 12, 43 and 44 are properly rejected under 35 U.S.C. § 112, first paragraph, even though (a) phosphoramidite chemistry is not essential material in the context of the present invention and (b) the specification provides evidence of the efficacy of the claimed method.

VII. ARGUMENT

A. The Claims Particularly Point Out and Distinctly Claim The Subject Matter Which Applicant Regards As The Invention

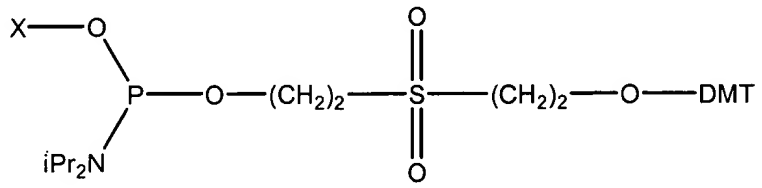
The Examiner has rejected claims 1-3, 6, 7, 9, 12, 43 and 44 under 35 U.S.C. § 112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Specifically, the Examiner states that it is not clear how a phosphoramidite (e.g., a compound of Formula II) can introduce a phosphate group. Formula II is as follows:



Claims 1-3, 6, 7, 12, 43 and 44

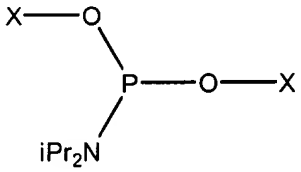
First, even assuming, *arguendo*, that the rejection is appropriate, the rejection should only be applied to claim 9, as the only recitation of a phosphoramidite in the claims under consideration occurs in claim 9. The text of claim 9 is as follows:

The method according to Claim 6, wherein said step *2)* of reacting said activated sites with a compound comprises reacting each of said activated sites with a compound selected from the group consisting of Formula I:



I

and Formula II:



II

wherein:

DMT is a dimethoxy trityl protecting group;

each X is a base-removable protecting group; and

iPr_2N is diisopropyl amino protecting group.

The Examiner provides no basis for rejecting the remaining claims, because there is no recitation of a phosphoramidite. The text of claim 1 is as follows:

A method for reducing non-specific binding of a molecule to an oligonucleotide array comprising a plurality of oligonucleotides on a surface of a solid support, wherein said surface has a plurality of designated regions and a plurality of protected regions, each of said plurality of protected regions having a protecting group thereon, said method comprising:

a) producing said plurality of oligonucleotides at each of said designated regions, each of said plurality of oligonucleotides having a terminal protecting group; and

b) replacing with a negatively charged phosphate residue, at least one of:

i) the protecting groups on each of said plurality of oligonucleotides produced in step *a)*, and

ii) the protecting groups on each of said plurality of protected regions; whereby non-specific binding of said molecule is reduced.

Thus, rejection of claims 1-3, 6, 7, 12, 43 and 44 is improper because the grounds for rejection, which rely on recitation of a phosphoramidite, do not apply to these claims. Applicants respectfully request that the Board consider claims 1-3, 6, 7, 12, 43 and 44 separately from claim 9 for purposes of appeal.

Claim 9

Second, the Examiner has not provided reasons why matter not included in the claims is *essential* to the invention. The material regarding the use of phosphoramidites is not "essential material" within the meaning of MPEP § 608.01(p). Essential material is defined therein to be material that is necessary to:

(1) Provide a written description of the claimed invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and set forth the best mode contemplated by the inventor of carrying out the invention as required by the first paragraph of 35 U.S.C. 112;

(2) Describe the claimed invention in terms that particularly point out and distinctly claim the invention as required by the second paragraph of 35 U.S.C. 112; or

(3) Describe the structure, material, or acts that correspond to a claimed means or step for performing a specified function as required by the sixth paragraph of 35 U.S.C. 112.

The material regarding the use of phosphoramidites is not essential material because it was well-known to the skilled artisan at the time of filing and therefore not needed either to describe the invention or to particularly point out and distinctly claim the invention. In fact, such material was so well-known that it is found in basic organic chemistry textbooks. As evidence, Applicants previously provided pages 1141-1144 of Organic Chemistry, 3rd Edition, by John McMurry (1992) as an exhibit (resubmitted here as part of Appendix B). On pages

1143-1144, the reaction of a phosphoramidite moiety with a hydroxyl moiety is shown, which produces a phosphite ester. The phosphite ester is subsequently oxidized to a phosphate ester with iodine. The phosphate ester is converted to a phosphate residue by reacting the phosphate ester with ammonia in the presence of water. This evidence demonstrates that phosphoramidite chemistry was well-known prior to the effective filing date of the instant application (indeed, more than 5 years prior to the effective filing date). Information which is well known in the art need not be described in detail in the specification (MPEP § 2163). For this reason, explicitly teaching or reciting each part of the process by which a phosphoramidite is converted into a phosphate is not necessary to provide a written description of the invention or to describe the claimed invention in terms that particularly point out and distinctly claim the invention. Thus, the particulars of phosphoramidite chemistry are not essential material.

Because the particulars of phosphoramidite chemistry are not essential material, MPEP § 2172.01 does not apply. As such, there is no basis for a rejection under 35 U.S.C. § 112, second paragraph. Withdrawal of the rejection is respectfully requested.

B. The Claims Are Enabled By The Specification

The rejection under 35 U.S.C. § 112, first paragraph, has two aspects. The first aspect of the rejection concerns the alleged omission from the claims of subject matter disclosed to be essential to the invention. The second aspect of the rejection concerns the specification allegedly not providing support for the claimed effect of reducing non-specific binding. Each aspect of the invention will be discussed separately below.

1. Phosphoramidite Chemistry Is Well-Known To One Of Skill In The Art And Need Not Be Described In The Instant Application

There is no need for the instant specification to show how to produce a negatively charged phosphate group by reacting an unprotected activated site with a phosphoramidite, because such reactions were well-known in the art as of the effective filing date. Moreover, as discussed above, the matter regarding phosphoramidite chemistry is not *essential* to the invention within the meaning of MPEP § 608.01(p). As such, MPEP § 2172.01 (incorrectly

cited in the Office Action as MPEP § 2171.01) is inapplicable. Thus, there is no basis for the first aspect of the instant rejection.

2. The Efficacy Of The Claimed Method Is Supported By Experimental Data

Claims 1-3, 6, 7, 9, 12, 43 and 44

The Examiner raises two issues with respect to the enablement of the method. First, the Examiner states that there is no data to support the claimed effect. Second, the Examiner states that replacing protecting groups with negatively charged groups would not have the intended effect on reducing non-specific binding. Applicants disagree. A discussion of each issue follows.

The instant application includes data in support of the claimed method. In Example 1, the control array includes a background where the protecting group remains attached to the support and the test array includes a background where the protecting group is replaced by a guanine nucleotide. As shown by claim 11 (currently withdrawn from consideration but within the scope of claim 1), guanine nucleotides are a type of negatively charged phosphate residue according to the present invention (when R_1 is a nucleoside moiety). Table 1 in Example 1 indicates that the background fluorescence intensity, which is due largely to non-specific binding, dropped from 348 to 53-61 upon replacing the protecting group with the phosphate-containing guanine nucleotide. In other words, the signal-to-noise ratio was improved six-fold or more when the protecting group on the support was replaced with a negatively charged phosphate residue. For this reason, the application as filed demonstrates that the claimed method reduces non-specific binding.

While the Examiner has repeatedly asserted that the addition of negative charges to an array will increase its affinity for positively charged molecules, this argument ignores the fact that the phosphate ester backbone of oligonucleotides in an array already include a series of negative charges. As such, it is unreasonable and unsupported to say that the addition of a single negative charge per activated site will have an appreciable effect on the array's affinity for positively charged molecules. Instead, the elimination of positively charged or hydrophobic protecting groups (see page 12, lines 13-20 of the specification) is believed to have the dominant

effect, because these groups are not part of a nucleic acid *in vivo*. The result of these modifications to the array is that there is essentially no change in molecules non-specifically attracted to the array by virtue of the array already having a substantial negative charge, but there would be far fewer molecules non-specifically attracted by virtue of the protecting groups. This means that there is a net decrease in the number of molecules of all types that non-specifically bind to an oligonucleotide array. It is further noted that the claimed method makes a synthetic oligonucleotide more closely resemble a nucleic acid found *in vivo*. Because proper functioning of a biological system depends upon specificity of interactions between nucleic acids and other molecules, it is advantageous to make an oligonucleotide on an array more closely resemble a nucleic acid found *in vivo*.

Claims 43 and 44

Moreover, the Examiner's arguments regarding charge do not apply to claims 43 and 44. Claims 43 and 44 are directed to reducing the non-specific binding of a nucleic acid to an oligonucleotide array. Nucleic acids typically do not include positively charged groups, so the introduction of negative charges to an array is not expected to increase non-specific binding of nucleic acids to an array. To the contrary, removal of positive charges is expected to *reduce* non-specific binding of nucleic acids to an oligonucleotide array. Accordingly, the Examiner has presented no reasoning as to why claims 43 and 44 are not enabled. Applicants respectfully request that the Board consider claims 43 and 44 separately for purpose of appeal.

VIII. CLAIMS

A copy of the claims involved in the present appeal is attached hereto as Appendix A. As indicated above, the claims in Appendix A do not include the amendments filed on February 6, 2006.

IX. EVIDENCE

A copy of evidence pursuant to §§ 1.130, 1.131, or 1.132 and/or evidence entered by or relied upon by the examiner that is relevant to this appeal is attached hereto as Appendix B. This evidence was included with the Amendment filed April 29, 2005 as Exhibit A.

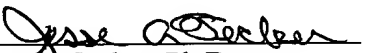
X. RELATED PROCEEDINGS

There are no related proceedings (see Section II). This is also indicated in Appendix C.

Applicants believe no additional fee is due with this response, however, if a fee is due, please charge our Deposit Account No. 18-1945, from which the undersigned is authorized to draw, under Order No. AFMX-P02-038.

Dated: August 28, 2006

Respectfully submitted,

By 

Jesse A. Fecker, Ph.D.
Registration No.: 52,883
ROPES & GRAY LLP
One International Place
Boston, Massachusetts 02110-2624
(617) 951-7000
(617) 951-7050 (Fax)
Attorneys/Agents For Applicant

APPENDIX A

Claims Involved in the Appeal of Application Serial No. 09/862571

1. A method for reducing non-specific binding of a molecule to an oligonucleotide array comprising a plurality of oligonucleotides on a surface of a solid support, wherein said surface has a plurality of designated regions and a plurality of protected regions, each of said plurality of protected regions having a protecting group thereon, said method comprising:

    a) producing said plurality of oligonucleotides at each of said designated regions, each of said plurality of oligonucleotides having a terminal protecting group; and

    b) replacing with a negatively charged phosphate residue, at least one of:

        i) the protecting groups on each of said plurality of oligonucleotides produced in step *a)*, and

        ii) the protecting groups on each of said plurality of protected regions; whereby non-specific binding of said molecule is reduced.

2. The method according to Claim 1, wherein said solid support comprises polymerized Langmuir Blodgett film, functionalized glass, germanium, silicon, polymers, (poly)tetrafluoroethylene, polystyrene, gallium arsenide, metal oxide films, and combinations thereof.

3. The method according to Claim 1, wherein said step *a)* of producing said plurality of oligonucleotides comprises:

1) attaching to each of said designated regions an independently selected linker monomer having a photolabile protecting group;

2) attaching an independently selected nucleotide monomer having a photolabile protecting group to each of said attached linker monomers using light directed methods to produce a plurality of attached monomers each having a terminal photolabile protecting group;

3) attaching an independently selected nucleotide monomer having a photolabile protecting group to each of said attached monomers using light directed methods to produce a plurality of oligonucleotides each having a terminal photolabile protecting group; and

4) repeating step *3)* from 0 to 119 times, to attach subsequent nucleotide monomers to each of said oligonucleotides produced in step *3)* to produce a plurality of oligonucleotides having a terminal photolabile protecting group.

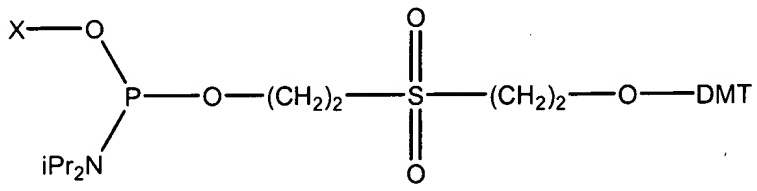
6. The method according to Claim 1, wherein said step *b)* of replacing comprises:

1) exposing at least one of: *i*) each of said plurality of oligonucleotides and *ii*) each of said plurality of protected regions, to an activator to remove the protecting groups, to produce activated sites; and

2) reacting said activated sites with one or more compounds that result in a negatively charged phosphate residue becoming bound to at least one of *i*) each of said plurality of oligonucleotides and *ii*) each of said plurality of protected regions.

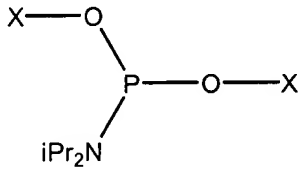
7. The method according to Claim 6, wherein said protecting group is a photolabile protecting group and said activator is selected from the group consisting of ion beams, electron beams, gamma rays, x-rays, ultra-violet radiation, light, infra-red radiation, microwaves, electric currents, radiowaves, and combinations thereof.

9. The method according to Claim 6, wherein said step *2)* of reacting said activated sites with a compound comprises reacting each of said activated sites with a compound selected from the group consisting of Formula I:



I

and Formula II:



II

wherein:

DMT is a dimethoxy trityl protecting group;

each X is a base-removable protecting group; and

iPr_2N is diisopropyl amino protecting group.

12. The method according to Claim 1, wherein said step *b)* of replacing comprises replacing both *i)* the protecting groups on each of said plurality of oligonucleotides produced in step *a)*, and *ii)* the protecting groups on each of said plurality of protected regions.

43. The method according to Claim 1, wherein non-specific binding of a nucleic acid polymer to an oligonucleotide array is reduced.

44. The method according to Claim 43, wherein the nucleic acid molecule is an oligonucleotide.

APPENDIX B

EXHIBIT A

Organic Chemistry

Third Edition

John McMurry
Cornell University

Brooks/Cole Publishing Company
Pacific Grove, California

Brooks/Cole Publishing Company
A Division of Wadsworth, Inc.

Printed in the United States of America

10 9 8 7 6 5 4 3 2

Library of Congress Cataloging in Publication Data

McMurry, John.
Organic chemistry / John McMurry. — 3rd ed.
p. cm.
Includes index.
ISBN 0-534-16218-5
1. Chemistry, Organic. I. Title.
QD251.2.M43 1992
547—dc20 91-40322
CIP

Sponsoring Editor: Harvey C. Pantzis
Editorial Assistant: Nancy Miaoulis
Production: Joan Marsh/Mary Douglas
Production Assistant: Scott Alkire
Manuscript Editor: Margaret Moore
Text Design: Nancy Benedict
Cover Design: Michael Rogondino
Cover Photo: MOZO Photo/Design
Chapter-Opener Illutrations: Pat Rogondino
Interior Illustration: John and Jean Foster, Dave Pauly
Typesetting: Jonathan Peck Typographers, Ltd.
Cover Printing: The Lehigh Press
Printing and Binding: R. R. Donnelley & Sons, Inc.
Photo, p. 1124: A. Lesk/SPL, Photo Researchers

Organizat

PROBLEM

29.21 Show the labeled cleavage products you would expect to obtain if the following DNA segment were subjected to each of the four cleavage reactions:

^{32}P-A-A-C-A-T-G-G-C-G-C-T-T-A-T-G-A-C-G-A

PROBLEM

29.22 Sketch what you would expect the gel electrophoresis pattern to look like if the DNA segment in Problem 29.21 were sequenced.

PROBLEM

29.23 Finish assigning the sequence to the gel electrophoresis pattern shown in Figure 29.17.

29.17 Laboratory Synthesis of DNA

The development of genetic engineering techniques in the last two decades brought with it an increased demand for efficient chemical methods for the synthesis of short DNA segments. Ideally, whole genes might be synthesized in the laboratory and inserted into the DNA of microorganisms, thereby directing the microorganisms to produce the specific protein coded for by that gene—perhaps insulin or some other valuable material.

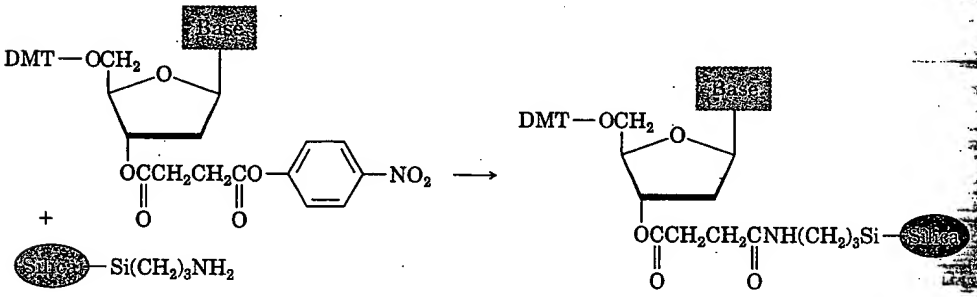
The problems of DNA synthesis are similar to those of protein synthesis (Section 27.11) but are considerably more difficult because of the structural complexity of the deoxyribonucleotide monomers. Each nucleotide has several reactive sites that must be selectively protected and deprotected at the proper times, and coupling of the four nucleotides must be carried out in the proper sequence. Despite these difficulties, some extremely impressive early achievements were recorded, most notably the synthesis by Khorana[4] in 1979 of the tyrosine suppressor tRNA gene from the bacterium *Escherichia coli*. Some 207 base pairs were assembled in an effort that required 10 years of work.

More recently, automated "gene machines" have become available, which allow the fast and reliable synthesis of DNA sequences 100–200 nucleotides long. These DNA synthesizers operate on a principle similar to that of the Merrifield solid-phase peptide synthesizer (Section 27.12). In essence, a protected nucleotide is covalently bound to a solid support, and one nucleotide at a time is added to the chain. When the last nucleotide has been added, the protecting groups are removed, and the synthetic DNA is cleaved from the solid support.

[4]Har Gobind Khorana (1922–); b. Raipur, India; Ph.D. University of Liverpool; professor, Massachusetts Institute of Technology; Nobel Prize in medicine (1968).

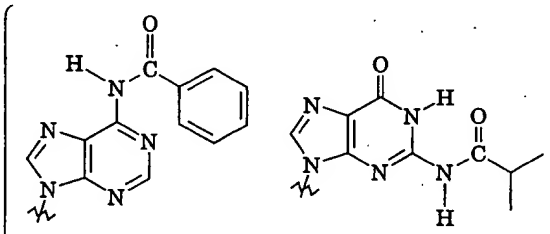
Step 1 in DNA synthesis involves attachment of a protected deoxynucleoside to a silica (SiO_2) support by an ester linkage to the deoxynucleoside's 3′ position. Both the 5′ hydroxyl and free amino groups on the heterocyclic bases must be protected to accomplish this attachment. Adenine and cytosine bases are protected by benzoyl groups, guanine is protected by an isobutyryl group, and thymine requires no protection. The deoxyribose 5′ hydroxyl is protected as its DMT ether, where DMT = *p*-dimethoxytrityl. Other protecting groups can also be used, but those mentioned here are common choices.

Step 1



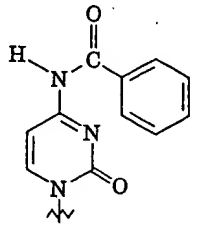
where DMT = CH_3O–(C)(Ph)(Ph)–OCH_3

Base =

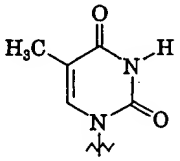


N-protected adenine

N-protected guanine



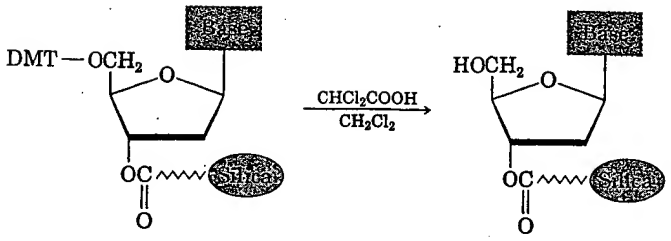
N-protected cytosine



Thymine

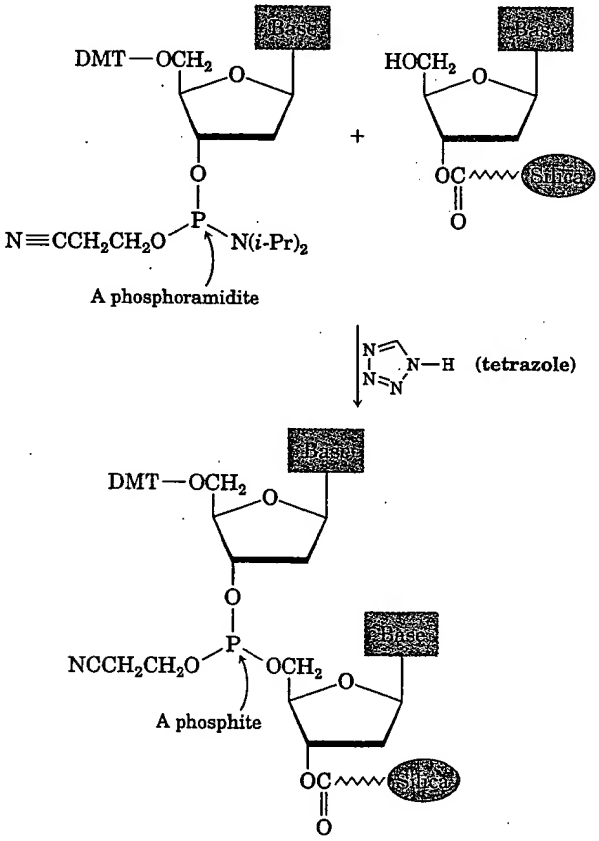
Step 2 involves deprotection of the 5′-deoxyribose hydroxyl by treatment with dichloroacetic acid in CH_2Cl_2 to remove the DMT group. The reaction proceeds rapidly by an S_N1 mechanism because of the stability of the tertiary, triply benzylic dimethoxytrityl cation.

Step 2



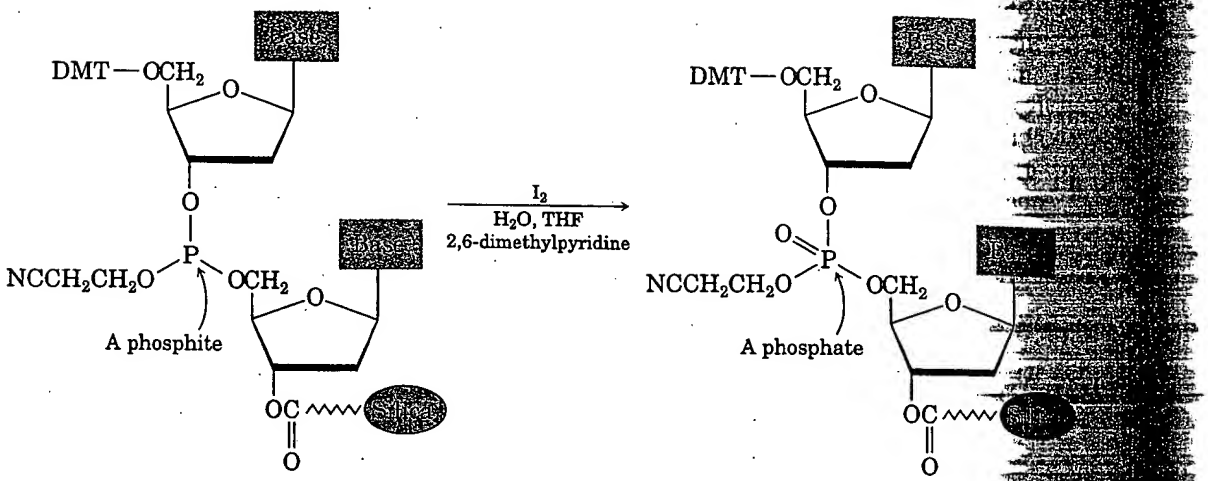
Step 3 involves coupling of the polymer-bonded deoxynucleoside with a protected deoxynucleoside containing a *phosphoramidite* group at its 3′ position. [A phosphoramidite has a trivalent phosphorus with the structure $R_2NP(OR)_2$.] The coupling reaction takes place in the polar aprotic solvent acetonitrile, requires catalysis by the heterocyclic amine tetrazole, and yields a *phosphite*, or trialkoxyphosphorus compound, $P(OR)_3$, as product. Note that one of the phosphorus oxygen atoms is protected by a β-cyanoethyl group. The coupling step takes place in better than 99% yield.

Step 3



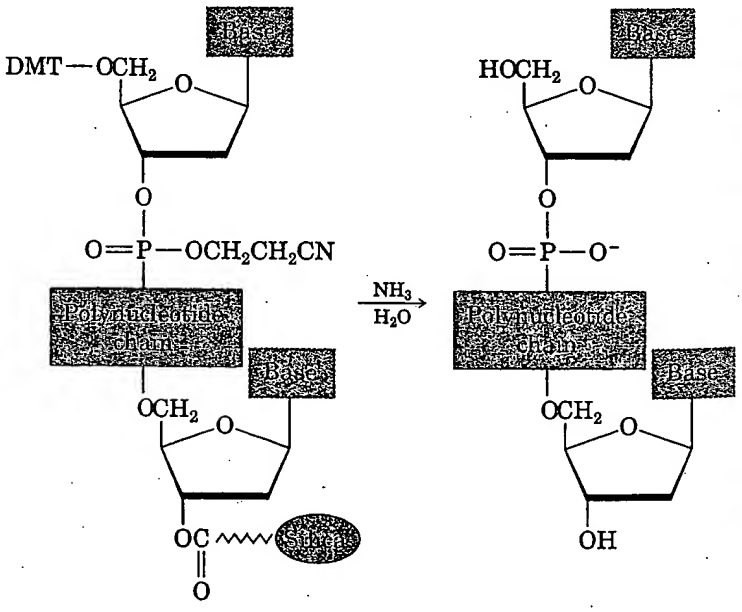
With the coupling completed, the phosphite product is then oxidized to a phosphate triester by treatment with iodine. The reaction is carried out in aqueous tetrahydrofuran in the presence of 2,6-dimethylpyridine.

Step 4



The cycle of steps—deprotection, coupling, and oxidation—is then repeated until a polydeoxyribonucleotide chain of the desired length and sequence has been built. The final step is to cleave all protecting groups from the heterocyclic bases and from the phosphates and to cleave the ester bond holding the DNA to the silica. All of these reactions are done at the same time by treatment with aqueous ammonia. Purification by electrophoresis then yields the synthetic DNA.

Step 5



PROBLEM

29.24 *p*-Dimethoxytrityl (DMT) ethers are easily cleaved by mild acid treatment. Show the mechanism, and suggest a reason why this ether cleavage is unusually easy.

PROBLEM

29.25 Propose a m
from the ph
reaction is o

29.18 Summary

A heterocy
of atom. Ni
heterocyclic
ally displa
urated hete
are the sin
usually sta
with electr
next to the
Pyridir
analog of b
trophilic ar
aromatic su
The nu
cleic acid)
organism's
acids yields
are constru
linked to C
in DNA), w
phate grou

The nucleot
nucleotide a
Molecu
by hydroge
coiled into a
gen bonds t
DNA are n

APPENDIX C

No related proceedings.